FOLLICULAR DEVELOPMENT, OOCYTE VIABILITY AND RECOVERY IN RELATION TO FOLLICULAR STEROIDS, PROLACTIN AND GLYCOSAMINOGLYCANS THROUGHOUT THE ESTROUS PERIOD IN SUPEROVULATED HEIFERS WITH A NORMAL LH SURGE, NO DETECTABLE LH SURGE, AND PROGESTIN INHIBITION OF LH SURGE[1]

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ABSTRACT

Estrous cycles of heifers (n = 137) were synchronized with prostaglandin ($PGF_{2\alpha}$) and follicular development stimulated with follicle stimulating hormone. Twenty-eight animals were administered Norgestomet implants 12 hr prior to the initial PGF2α injection to suppress the LH surge that initiates ovulation. Animals were ovariectomized every 12 hr after the initial PGF2α (7–9/time, 12–108 hr and at 192 and 240 hr post PGF2α) and divided into three treatment groups to consist of: 1) animals exhibiting a normal luteinizing hormone (LH) surge (n = 86), 2) animals in which no LH surge was detected (n = 23), and 3) suppression of the LH surge via Norgestomet implants (72–108 hr, n = 28). Follicular diameter was measured and follicular fluid was collected for analysis of prolactin, estradiol, progesterone and glycosaminoglycan concentrations. Progesterone concentrations were increased in animals exhibiting an LH surge as compared to animals in which no LH surge was detected; primarily in large follicles (> 8 mm diameter) after the LH surge. Animals not exhibiting an LH surge also had increased follicular progesterone concentrations compared to Norgestomet-implanted animals (242.3 ± 36.3 *vs* 86.7 ± 6.4 ng/ml, respectively, $P < .01$), indicating some LH stimulation. Follicular estradiol in animals exhibiting an LH surge increased up to the time of LH surge detection and then declined whereas animals with no LH surge detected had follicular estradiol concentrations that declined after the $PGF_{2\alpha}$ injection. No differences were noted between those that did not exhibit an LH surge or in which the LH surge was suppressed with Norgestomet in relation to follicular estradiol concentrations. Follicular estradiol concentrations increased with follicular size in all treatment groups ($P < .01$). Follicular concentrations of prolactin were increased in small follicles ($P < .05$; ≤ 4 mm diameter) and follicular prolactin increased from 12 to 36 hr post PGF2α injection, then declined after the LH surge. Follicular glycosaminoglycan concentrations decreased with increases in follicular size ($P < .01$) and were higher in animals that did not exhibit an LH surge ($P < .01$). No differences in follicular glycosaminoglycans were noted between Norgestomet-implanted animals and those not exhibiting an LH surge. In the animals representing days 4 and 6 of the subsequent estrous cycle (192 and 240 hr post PGF2α), numbers of small-sized follicles were increased. Follicular progesterone and estradiol concentrations were related to atretic large follicles unovulated from the prior estrus and a new wave of growth in small and medium follicles. Follicular prolactin and glycosaminoglycans increased with time of the new estrous cycle and were increased in smaller follicles ($P < .01$). Suppression of LH with progestin implants (Norgestomet) may relate to early effects of progesterone, which may not be totally eliminated at target tissues and subsequently alters the LH surge, steroidogenesis of the follicle, and ovulation. Oocytes were predominantly found in the follicular fluid from animals in which an LH surge was detected and in the buffer wash of follicles in which no LH surge was detected. Oocyte viability was higher in animals exhibiting an LH surge (75% viable) whereas the oocytes of Norgestomet-implanted animals were 75% degenerate.

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INTRODUCTION

The maximum utilization of in vitro fertilization and subsequent embryo transplantation in meat animals has been limited due to biological rather than technical reasons. Limited understanding of the mechanisms that control follicular development, oocyte maturation, and ovulation has been primarily responsible for the variable results in relation to superovulatory response and small numbers of mature, fertilizable oocytes collected (1–4). Elucidation of many new ovarian hormonal growth factors (IGF-I, FGF, EGF, inhibins, oxytocin), immunological hormones (interleukins, thymosins, cytokines, tumour necrosis factors), and follicular binding proteins (follistatin, insulin-like growth factor binding proteins, heparin binding growth factor) have added to the complexity of events associated with follicle selection, growth, and ovulation.

Glycosaminoglycans are important in the development of cell growth, differentiation and adhesion (5) and can modulate follicle stimulating hormone (FSH) and luteinizing hormone (LH) receptor responses (6–8). More recently, glycosaminoglycans are being viewed as important binding proteins for growth factors (heparin; 9) and cholesterol esters for internalization in the production of gonadal steroids (10). Low concentrations of glycosaminoglycans are associated with increased follicular estrogen concentrations (11, 12) and low progesterone content (13, 14) but increased glycosaminoglycans relate to the follicular atresia (13).

The modulatory roles of prolactin on follicular steroid production is well established in vitro (15–19) and mRNA for prolactin receptor has been detected in granulosa cells (20), but its major site of regulation (pituitary or ovary) is still poorly understood, particularly in farm animals.

Much of the final aspects of follicular development and secretion depend on luteinizing hormone to induce steroidogenic activity, ovulation, and transformation of granulosa to the luteal cells of the corpus luteum. The superovulated model to study follicular development seems to be typical enough to the development and ovulation of the follicle associated with the normal process (21) to be a relevant model and has the important advantage of providing considerably more follicles to study. The objectives of this investigation were to 1) monitor differences in follicular development during the estrual period in superovulated heifers which had a normal LH surge, no detectable LH surge, and in which the LH surge was suppressed with Norgestomet implant (progestin), 2) define the follicular size and developmental time relationships with follicular progesterone, estradiol, prolactin, and glycosaminoglycans with the above treatments, 3) determine recovery and viability of oocytes in relation to the above parameters, and 4) monitor ovarian, follicular hormonal and oocyte viability in the early stages of the subsequent estrous cycle (days 2, 4, 6).

MATERIALS AND METHODS

Cycling crossbred beef heifers (16 to 18 months of age; n = 137) were synchronized to estrus with prostaglandin ($PGF_{2\alpha}$; Lutalyase, UpJohn Co., Kalamazoo, MI) and superovulated with FSH-P (i.m., Burns Biotech, Omaha, NE). Animals with a palpable corpus luteum were initially administered $PGF_{2\alpha}$ (25 mg), then on days 9 to 12 of the estrous cycle, injections of FSH-P were administered for 4 d (6, 3, 2, and 2 mg twice daily). Heifers with a palpable corpus luteum were injected in three 10-mg doses of PGF2α beginning 60 hr after the initial FSH injection (time 0, 12, 24 hr; Figure 1). Animals were divided into three treatment groups to consist of animals that exhibited a normal LH surge (n = 86), animals in which no LH surge was detected in the peripheral serum samples (n = 23) and animals in which the LH surge was suppressed with Norgestomet implants (n = 28; Synchromate-B, CEVA, Overland Park, KS). Norgestomet was ear implanted at the

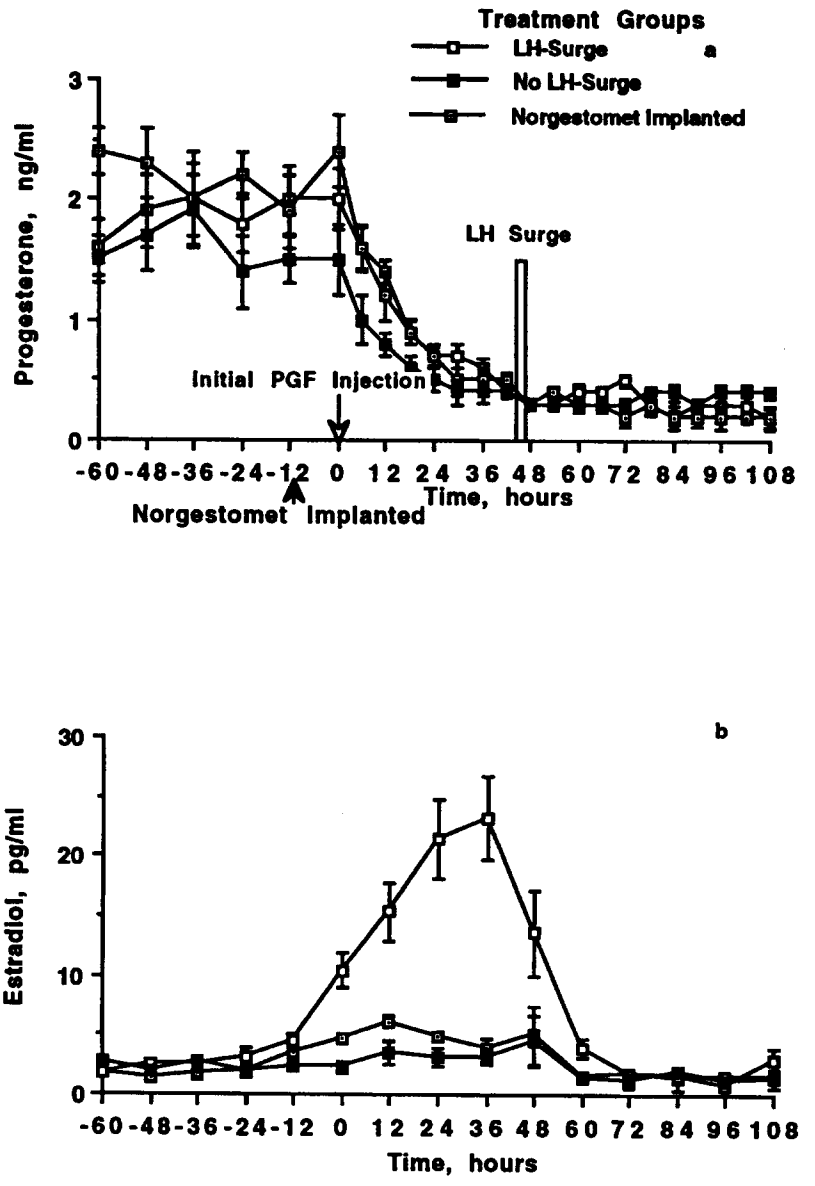

Figure 1. Changes in peripheral serum progesterone (top panel, a) and estradiol (lower panel, b) in animals exhibiting an ovulatory LH surge, no detectable LH surge, or in which the LH surge was inhibited with a progestin implant (Norgestomet). No differences were noted in the decline of progesterone concentrations that accompanied corpus luteum regression between the three animal groups but estradiol concentrations were significantly increased ($P < .01$) in animals exhibiting an LH surge (Figure 1b).

fifth FSH injection (-12 hr; n = 28; Figure 1) and animals were later injected with $PGF_{2\alpha}$ (time 0, 12, 24 hr; Figure 1). Blood was sampled from animals (via the tail vein) at 12-hr intervals until the initial $PGF_{2\alpha}$ injection (-60 to 0 hr; Figure 1) and thereafter every 6 hr to 108 hr for analysis of serum progesterone, estradiol, and LH.

Animals were ovariectomized every 12 hr after the initial $PGF_{2\alpha}$ (n = 7 to 9/time; 12 to 108 hr, then at 192 and 240 hr post $PGF_{2\alpha}$, n = 34). Follicles were analyzed into the beginning of next estrous cycle (192, 240 hr post $PGF_{2\alpha}$) as a negative control as these follicles left unovulated should be atretic and oocyte quality poor. Animals receiving

Norgestomet implants were ovariectomized 72, 84, 96, and 108 hr post $PGF_{2\alpha}$ and implants remained in the animal until removed at ovariectomy. Ovaries were weighed, ovulation points counted, and follicular diameters measured. In follicles greater than 4 mm diameter, follicular fluid was collected and each follicle flushed with heparinized, phosphate-buffered saline (pH 7.4). In follicles 4 mm or smaller in diameter, follicular fluid was collected and pooled. Follicular fluid was centrifuged and frozen after oocytes were removed.

Oocyte Recovery and Viability. In follicles > 4 mm diameter, follicular fluid was collected in either a 1- or 5-cc syringe and then the follicle was flushed with a comparable volume of heparinized phosphate buffered saline (PBS). In follicles ≤ 4 mm in diameter, follicular fluid was pooled for each ovary and the syringe rinsed with heparinized PBS to collect oocytes.

Oocytes were initially identified in either follicular fluid or the heparinized PBS rinse and evaluated under a dissecting microscope. Oocytes were then fixed with acetic acid:methanol (1:3) and stained with 1% orecin stain in 40% acetic acid for examination under phase contrast optics. Oocyte classification was similar to the method of Leibfried and First (22) and Veek (23).

Assays. Concentrations of LH were measured in serum to determine the period of the LH surge (24) and differentiate treatment groups. Serum progesterone concentrations were measured to monitor luteal regression and measured by radioimmunoassay (RIA; 21, 25). Serum estradiol concentrations were measured by RIA to monitor follicular development (21, 25).

Estradiol and progesterone were measured in follicular fluid after diluted to 1:100 in PBS (21). Prolactin (20 μl follicular fluid) was measured by the method of Klindt et al. (26) as validated for bovine follicular fluid. Concentrations of glycosaminoglycans (5 μl follicular fluid) were measured by Alcian Blue method of Kubajak and Ax (27). The intra- and inter-assay variations for LH, estradiol, and progesterone were 3.1% and 11.7%, 7.0% and 16.0%, and 7.2% and 10.9%, respectively. Intra- and inter-assay variation for prolactin was 1.1% and 3.5% and for total glycosaminoglycans was 3.4% and 5.2%.

Statistical Analysis. Hormonal changes in peripheral circulation were analyzed for a split-plot, completely randomized design (for repeated measurements over time; 28) with animal (treatment) used to test for differences in main effects. Follicular hormonal and oocyte data were analyzed in a similar fashion (repeated measurements within animal) but animal(treatment × time post $PGF_{2\alpha}$) was used as the error term for differences in main effects. Time after $PGF_{2\alpha}$ was analyzed in two models for follicular responses that consisted of 0 to 108 hr post $PGF_{2\alpha}$ and 48 to 108 hr post $PGF_{2\alpha}$ because the LH surge occurred after 36 hr post $PGF_{2\alpha}$. No LH surges were detected until the 48-hr sampling; thus from 0 to 36 hr post $PGF_{2\alpha}$, LH and no LH surge treatment groups could not be differentiated for follicular response differences. Analysis of viability or recovery of oocytes were by Chi-square.

RESULTS

Peripheral Serum—Progesterone, Estradiol, and LH During the Estrual Period. Seventy-nine percent of the non-implanted animals exhibited an LH surge (41.8 ± .6 hr post $PGF_{2\alpha}$) as detected in peripheral serum from the 6-hr samplings (0 to 108 hr post $PGF_{2\alpha}$). Serum progesterone concentrations reveal that all animals had a regression of corpus luteum function after the $PGF_{2\alpha}$ injection (time 0, Figure 1a). Serum estradiol concentrations were considerably increased in animals exhibiting an LH surge (Figure 1b), whereas animals in which no LH surge was detected or the LH surge was suppressed with Norgestomet implants had lower estradiol concentrations ($P < .01$; Figure 1b). None of the animals implanted with Norgestomet exhibited an LH surge.

TABLE 1. OVARIAN CHANGES DURING THE ESTRUAL PERIOD AND EARLY STAGES OF THE NEXT ESTROUS CYCLE.

	Hours post $PGF_{2\alpha}$ injection										
	12	24	36[a]	48	60	72	84 (1)[a]	96 (1)	108 (2)	192 (4)	240 (6)
Ovarian weight[b], g											
LH Surge	30.0 ± 4.0	32.9 ± 4.0	28.3 ± 4.0	38.9 ± 4.7	41.3 ± 5.3[c]	21.5 ± 4.0	21.1 ± 4.3	29.8 ± 4.3	17.6 ± 6.1	35.4 ± 14.6	59.1 ± 4.8[c]
No detectable LH surge	30.0 ± 4.0	32.9 ± 4.0	28.3 ± 4.0	26.5 ± 7.6	15.9 ± 6.2	15.8 ± 6.2	16.4 ± 5.3	20.6 ± 4.3	14.4 ± 5.3	—	22.6 ± 1.6
Norgestomet-implanted						20.3 ± 4.0	20.4 ± 4.0	18.1 ± 4.0	22.6 ± 4.0	—	—
Corpus luteum weight[b], g											
LH surge	4.47 ± .34	4.33 ± .34	3.31 ± .34	2.63 ± .40	2.01 ± .52	1.69 ± .34	1.44 ± .36	1.80 ± .37	1.70 ± .36	—	—
No detectable LH surge	4.47 ± .34	4.33 ± .34	3.31 ± .4	3.05 ± .64	2.03 ± .45	2.03 ± .52	1.33 ± .45	1.89 ± .45	1.51 ± .45	—	—
Norgestomet-implanted						1.54 ± .34	1.01 ± .34	1.28 ± .34	1.13 ± .34	—	—
Ovulation points[b]											
LH surge	.14 ± 2.5	0.0	.28 ± 2.4	.8 ± 2.9	5.25 ± 3.3	13.8 ± 2.4	22.7 ± 2.7	32.0 ± 2.7	10.3 ± 2.7	7.8 ± 6.1	19.0 ± 2.9
No detectable LH surge	—	—	—	—	—	—	—	—	—	—	—
Norgestomet-implanted						—	—	—	—	—	—
Follicles ≤ 4 mm diameter[b]											
LH surge	7.4 ± 5.0	14.1 ± 4.9	14.7 ± 4.9	11.6 ± 5.9	24.2 ± 6.5	20.8 ± 4.9	16.3 ± 5.4	31.8 ± 5.4	12.3 ± 5.4	33.3 ± 13.3	55.3 ± 6.3
No detectable LH surge	7.4 ± 5.0	14.1 ± 4.9	14.7 ± 4.9	8.5 ± 9.3	17.3 ± 7.6	16.6 ± 7.6	16.7 ± 6.6	14.5 ± 6.6	20.2 ± 6.5	—	68.3 ± 18.6
Norgestomet-implanted						26.3 ± 7.6	26.1 ± 7.6	30.6 ± 7.6	38.8 ± 7.6	—	—
Follicles > 4 and ≤ 8 mm diameter[b]											
LH surge	9.3 ± 1.5	6.8 ± 1.5	4.8 ± 1.5	4.8 ± 1.7	7.5 ± 1.9	2.6 ± 1.5	5.3 ± 1.6	2.7 ± 1.6	3.8 ± 1.6	2.8 ± 1.2	1.6 ± .3
No detectable LH surge	9.3 ± 1.5	6.8 ± 1.5	4.8 ± 1.5	4.5 ± 2.7	2.7 ± 2.3	4.7 ± 2.3	3.0 ± 1.9	3.0 ± 1.9	7.2 ± 1.9	—	2.5 ± 2.1
Norgestomet-implanted						6.0 ± 1.3	4.7 ± 1.3	6.8 ± 1.3	6.7 ± 1.3	—	—
Follicles > 8 mm diameter[b]											
LH surge	18.0 ± 2.6	19.3 ± 2.6	15.7 ± 2.6	19.0 ± 3.1	25.0 ± 3.4[c]	4.4 ± 2.6[d]	3.3 ± 2.8[d]	4.6 ± 2.8[d]	2.6 ± 2.8[c]	1.5 ± .6	2.6 ± .5
No detectable LH surge	18.0 ± 2.6	19.3 ± 2.6	15.7 ± 2.6	11.5 ± 4.9	11.0 ± 4.0	7.6 ± 4.0	8.0 ± 3.5	12.2 ± 3.5	7.5 ± 3.5	—	6.0 ± 2.9
Norgestomet-implanted						11.6 ± 2.3	13.0 ± 2.3	12.0 ± 2.3	15.4 ± 2.3	—	—

Mean ± SE

[a]12 to 36 hr post $PGF_{2\alpha}$ animals exhibiting or not exhibiting an LH surge cannot be differentiated. Probable days of subsequent cycle (1 to 6).

[b]Changed over time ($P < .05$).

[c]Different between animals with no detectable LH surge and animals exhibiting an LH surge ($P < .05$).

[d]Different between animals exhibiting an LH surge and Norgestomet-implanted animals ($P < .05$).

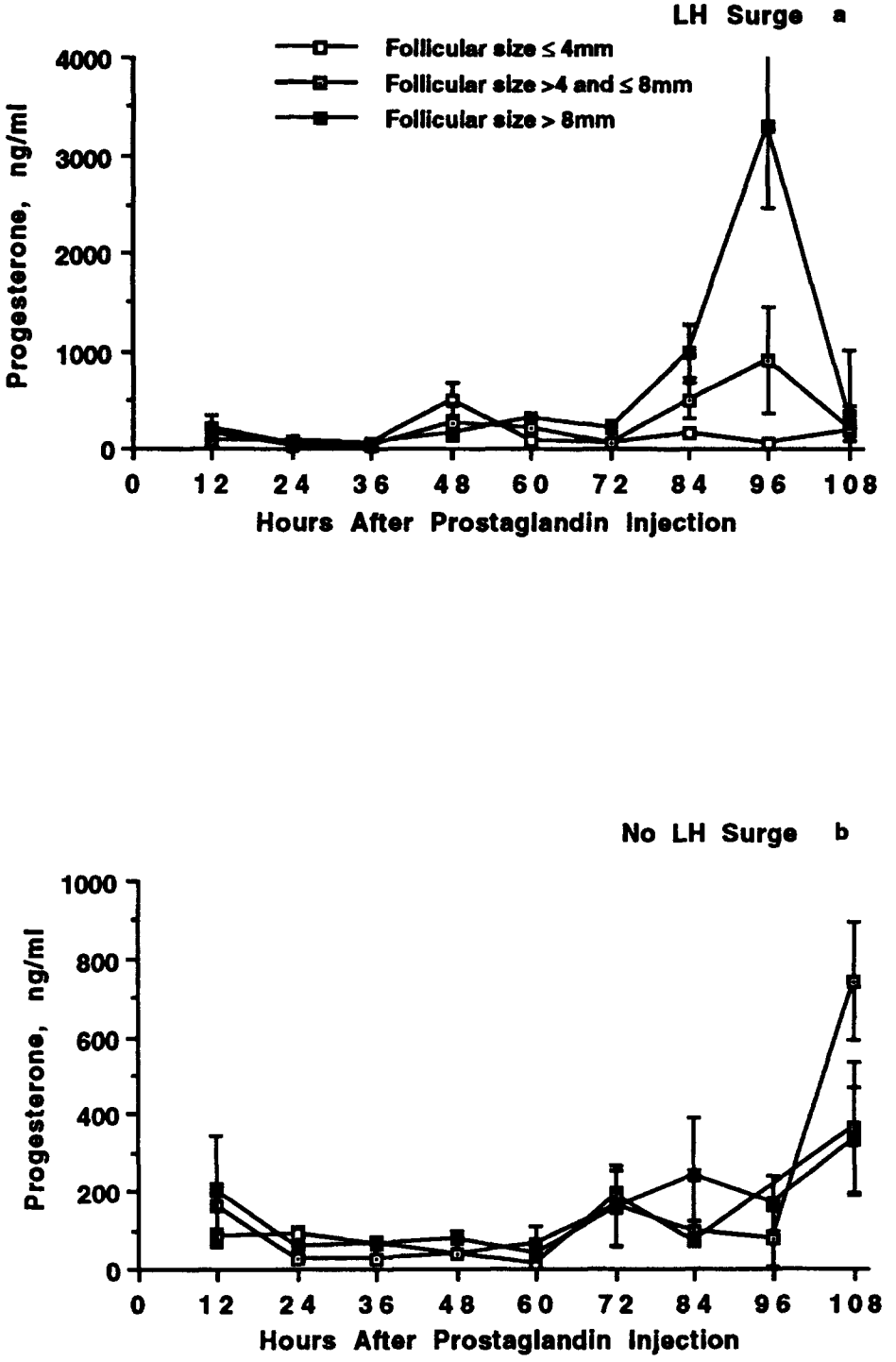

Figure 2. Changes in follicular progesterone concentrations (≤ 4, > 4 and ≤ 8, and > 8 mm diameter) in animals exhibiting an LH surge (top panel, a) and those in which no LH surge was detected (lower panel, b). Follicular progesterone concentrations from animals exhibiting an LH surge were increased overall and after the LH surge ($\bar{x}$ = 42 hr; $P < .05$). Large follicles had increased concentrations of progesterone ($P < .05$).

Ovarian Changes During the Estrual Period. No differences were noted between treatments in relation to regressing corpus luteum weights (Table 1). Total follicles classifed and analyzed from animals exhibiting an LH surge were 1073, 1128 from animals not exhibiting an LH surge, and 799 from animals in which the Norgestomet implants inhibited the LH surge. Ovarian weights were increased in animals exhibiting an LH surge (Table 1), initially from increased follicular activity (i.e., 60 hr) and subsequently from developing corpus luteum (240 hr). Prior to the initiation of ovulation, follicle numbers (> 8 mm diameter) were increased in animals exhibiting an LH surge (60 hr); but after the period of ovulation in animals exhibiting an LH surge, those not exhibiting an LH surge tended to show increased number of unovulated large follicles (72 to 240 hr; Table 1).

Follicular Hormones. Follicular fluid concentrations of progesterone were increased in animals exhibiting an LH surge (note scale difference, Figure 2a), which was primarily represented in large follicles ($P < .05$; > 8 mm diameter) after the LH surge (Figure 2a). Follicular fluid progesterone concentrations increased over time post PGF2α in both treatment groups (Figure 2). Comparison of follicular progesterone concentrations between animals not exhibiting an LH surge and those in which the LH surge was suppressed with a Norgestomet implant showed increased progesterone concentrations in each follicular size group in animals not exhibiting an LH surge (no detected LH surge *vs* Norgestomet implanted, respectively; 242.3 ± 36.3 *vs* 86.7 ± 6.4 ng/ml; $P < .01$), but no differences were noted between the two treatments over time post $PGF_{2\alpha}$ (Table 2).

Follicular estradiol concentrations in animals exhibiting an LH surge (Figure 3a) increased up to the time of LH surge detection ($\bar{x}$ = 42 hr), then subsequently declined. In animals with no detectable LH surge (Figure 3b), follicular estradiol concentrations declined subsequent to the $PGF_{2\alpha}$ injection. Follicular fluid concentrations of estradiol were higher in animals in which an LH surge was exhibited; and in both groups, estradiol increased with follicular size ($P < .01$; Figures 3a and 3b). No differences were detected in follicular estradiol concentrations between Norgestomet-implanted animals or in animals without a detectable LH surge (Table 2), but estradiol tended to increase with follicular size ($P < .10$).

Overall, follicular prolactin concentrations were increased in smaller follicles and concentrations decreased with follicular size (small = 11.2 ± .45, medium = 10.3 ± .38, large = 9.7 ± .34; $P < .01$). Follicular prolactin increased 12 to 36 hr post-PGF2α injection and subsequently declined after the time of the LH surge (Figure 4; $P < .05$). No major differences were noted in follicular prolactin concentrations between treatments (LH surge *vs* no detectable LH surge; Figures 4a and 4b). Follicular prolactin concentrations were not different between animals that had no detectable LH surge and Norgestomet implanted animals.

Follicular concentrations of glycosaminoglycans between follicular sizes and time after the $PGF_{2\alpha}$ injection are depicted in Figure 5. Glycosaminoglycans decreased with increases in follicular size ($P < .01$) and were higher in animals that did not exhibit an LH surge ($P < .01$), primarily in the small- (≤ 4 mm) and medium-sized (> 4 and ≤ 8 mm) follicles (Figure 5b). In animals that had no detectable LH surge or the LH surge was inhibited with an Norgestomet implant, glycosaminoglycans decreased with increases in follicular size (Table 2) but there were no differences between the two treatments. Changes in glycosaminoglycans in small follicles were curvilinear whereas linear in medium and large size follicles (Figure 5; $P < .01$).

Follicular/Hormonal Changes During the Subsequent Estrous Cycle (days 1 to 6). Large unovulated follicles were noted in both response groups (LH surge and no detectable LH surge) four to six d into the next estrous cycle (Table 1). Follicular numbers increased over time particularly in the small-size (≤ 4 mm diameter) group ($P < .01$;

TABLE 2. CHANGES IN FOLLICULAR HORMONES AND GLYCOSAMINOGLYCANS IN ANIMALS NOT EXHIBITING AN LH SURGE (N = 15) AND ANIMALS IN WHICH A PROGESTIN (NORGESTOMET) IMPLANT PRIOR TO PROSTAGLANDIN ($PGF_{2\alpha}$) INJECTION INHIBITED THE LH SURGE (N = 28).

Treatment time	No detectable LH surge			Norgestomet implanted				
post $PGF_{2\alpha}$, hr	72 (46)[a]	84 (50)	96 (53)	108 (44)	72 (179)	84 (153)	96 (181)	108 (196)
Follicular progesterone, ng/ml								
≤ 4 mm[b]	[d]185.7 ± 24.6[c,e]	69.5 ± 11.5	—	362.0 ± 169.0[e]	[d]54.7 ± 13.0[f]	67.3 ± 7.0[e]	136.9 ± 9.8[e]	145.5 ± 8.3[e,f]
> 4 and ≤ 8 mm	[d]161.1 ± 102.3	95.9 ± 63.0	75.9 ± 41.6[e]	743.3 ± 150.0	[d]36.4 ± 5.1	19.7 ± 3.2[f]	86.5 ± 3.7	50.0 ± 3.9[f]
> 8 mm	[d]154.7 ± 97.6	240.3 ± 147.8	170.2 ± 71.1	329.3 ± 140.0	[d]64.5 ± 18.4	86.1 ± 31.1	132.5 ± 37.3	115.6 ± 32.5
Follicular estradiol, ng/ml								
≤ 4 mm	.14 ± .01[e]	.82 ± .09[e]	—	4.2 ± .01	[d]1.2 ± .05[f]	1.8 ± .48[e]	1.5 ± .17[e]	1.4 ± .08[e]
> 4 and ≤ 8 mm	[d]2.8 ± .60	.50 ± .28	.29 ± .22	.52 ± .11	[d]1.8 ± .25[f]	1.9 ± .34[e]	.48 ± .13	.4 ± .08
> 8 mm[b]	[d]13.8 ± .70	6.1 ± .70	2.4 ± .16	.6 ± .16	[d]2.9 ± .19[f]	2.7 ± .27	.54 ± .09	.63 ± .07
Follicular prolactin, ng/ml								
≤ 4 mm	7.1 ± 1.1	17.0 ± 1.0[e]	7.5 ± 3.4	6.3 ± 1.1[e]	7.5 ± 1.1[e]	8.8 ± 1.1[e]	8.0 ± 1.1[e]	8.2 ± 1.0[e,f]
> 4 and ≤ 8 mm	[d]7.5 ± 1.0	10.8 ± 1.1	7.5 ± 1.1	6.7 ± 1.1	[d]7.1 ± 1.0	6.9 ± 1.0[f]	5.0 ± 1.1[f]	6.4 ± 1.0
> 8 mm[b]	[d]7.7 ± 1.1	7.3 ± 1.0	5.9 ± 1.0	4.5 ± 1.1	5.8 ± 1.0[f]	6.6 ± 1.1	6.1 ± 1.0	5.8 ± 1.0
Follicular glycosaminoglycans, μg/ml								
≤ 4 mm	1827.2 ± 25.9[e]	1205.9 ± 142.0	—	1652.2 ± 305.0	[d]1760.4 ± 44.0[e,f]	1531.4 ± 29.3[e]	1481.5 ± 21.2[e]	1799.4 ± 66.9[e,f]
> 4 and ≤ 8 mm	[d]1318.6 ± 15.0	1231.2 ± 150.0	1026.1 ± 86.6	1210.1 ± 94.5	1141.8 ± 37.5[f]	997.5 ± 30.3	965.8 ± 25.5	1132.6 ± 24.5
> 8 mm	1027.7 ± 81.8	1028.5 ± 56.0	933.2 ± 23.0	939.7 ± 79.0	943.0 ± 31.1[f]	914.1 ± 21.3[f]	998.2 ± 22.0	991.5 ± 20.0[f]

[a]Time post $PGF_{2\alpha}$ and numbers of follicles sampled ( ).
[b]Follicular size, mm diameter.
[c]Mean ± SE.
[d]Significantly changed with time post $PGF_{2\alpha}$.
[e]Significantly changed with follicular size.
[f]Significantly different between treatments for comparable time/size.

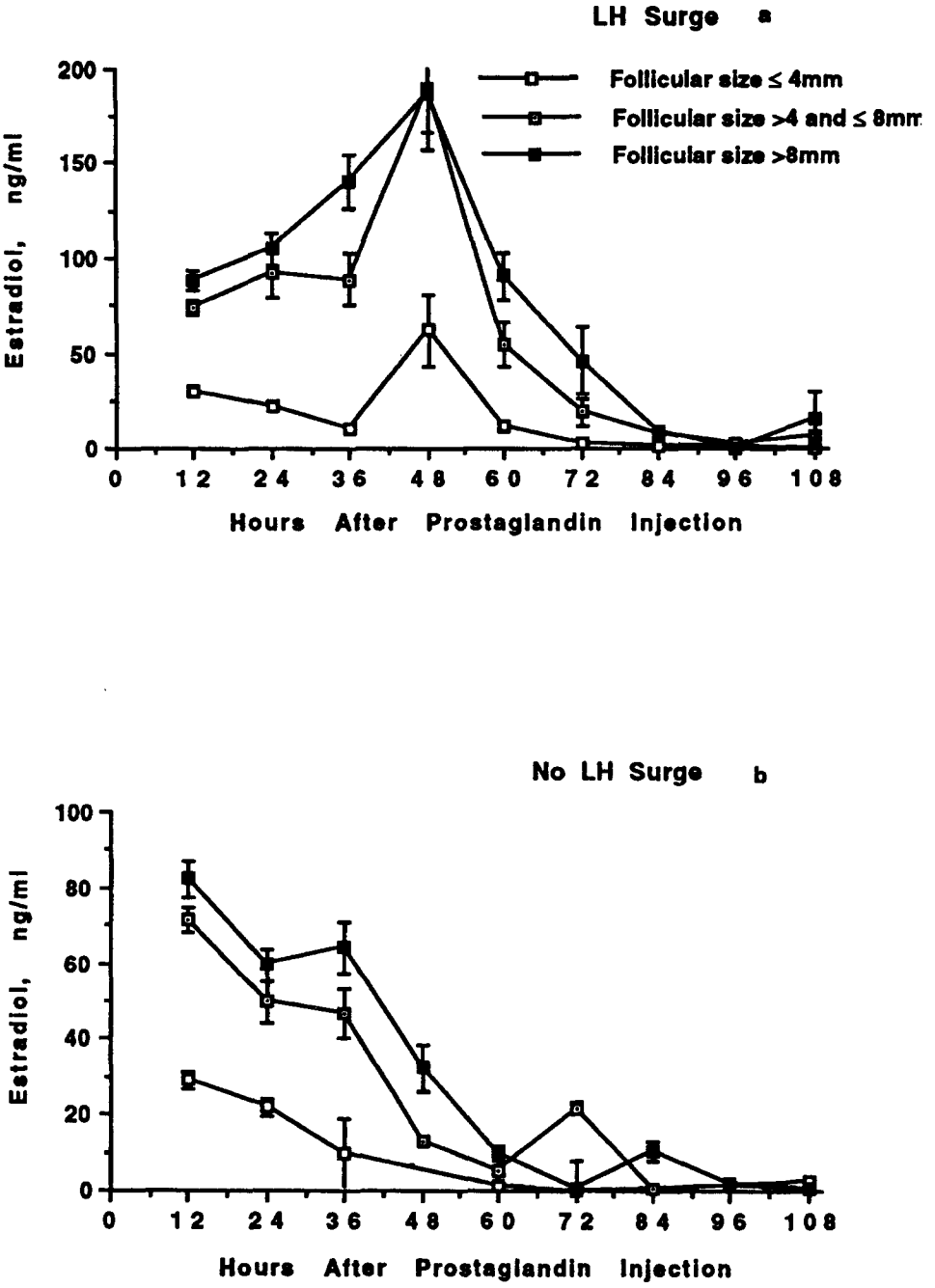

Figure 3. Follicular estradiol changes in animals exhibiting an LH surge (top panel, a) and those not exhibiting an LH surge (lower panel, b). In animals exhibiting an LH surge, follicular estradiol concentrations increased up to the time of the LH surge ($\bar{x}$ = 42 hr) and follicular estradiol concentrations were greater than concentrations from follicles of animals not exhibiting an LH surge ($P < .01$). Follicular estradiol concentrations increased with follicular size (diameter) in both groups ($P < .01$).

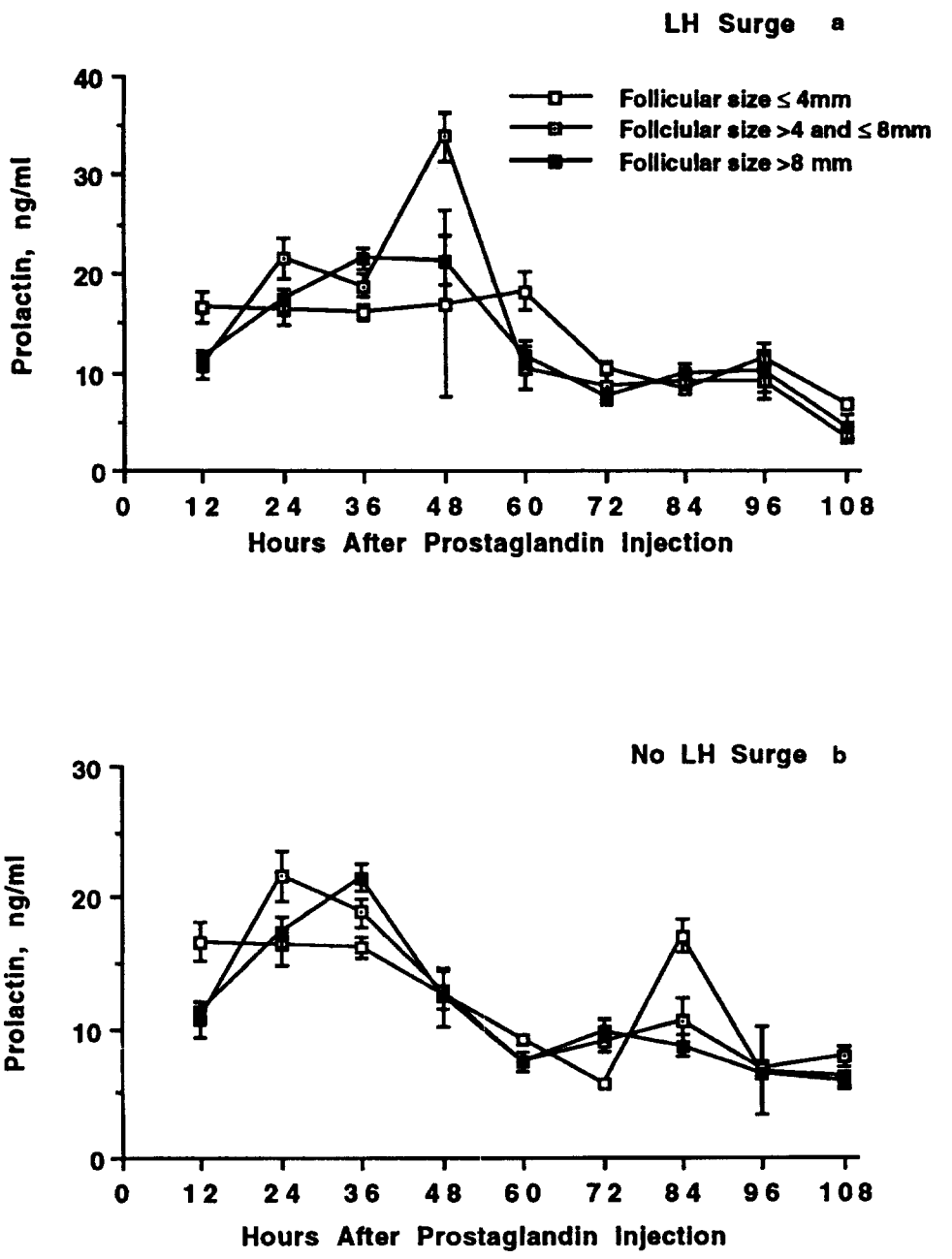

Figure 4. Changes in follicular prolactin concentrations (≤ 4, > 4 and ≤ 8, and > 8 mm diameter) in animals exhibiting an LH surge (top panel, a) and those not exhibiting an LH surge (lower panel, b). Follicular prolactin concentrations decreased in both animal groups after the prostaglandin injection (Time 0; $P < .05$) and was increased in small follicles (≤ 4 mm diameter; $P < .01$). No major differences were noted between the two animal groups (LH surge/no detectable LH surge).

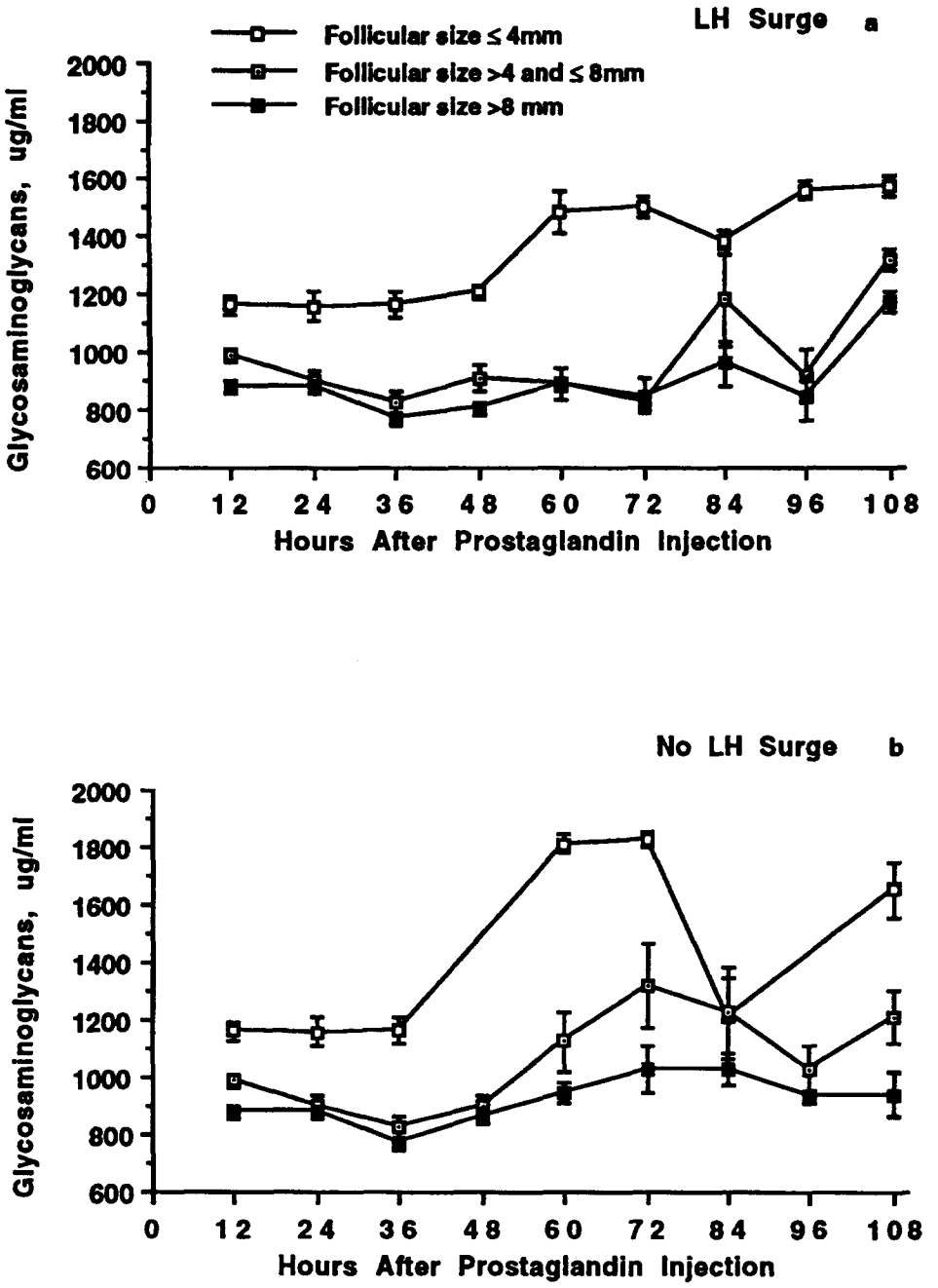

Figure 5. Follicular glycosaminoglycan changes in animals exhibiting an LH surge (top panel, a) and those not exhibiting an LH surge (lower panel, b). Overall concentrations of glycosaminoglycans were increased in animals not exhibiting an LH surge, primarily in the small- (≤ 4 mm) and medium-sized (> 4 and ≤ 8 mm) follicles. Glycosaminoglycans decreased in concentration with increases in follicular size in both animal groups ($P < .01$).

days 4 and 6 of subsequent cycle; Table 1). Differences between treatments were primarily in the large follicular size group (> 8 mm diameter) in which animals not exhibiting an LH surge had a residual group of unovulated follicles still on the ovaries through the post ovulatory period ($P < .05$).

Follicular progesterone concentrations (all follicles) were higher for animals exhibiting an LH surge (132.4 ± .2 *vs* 70.9 ± .3; $P < .05$) as compared to animals without a detectable LH surge. Significant size differences in relation to follicular progesterone concentrations were also detected (small, 89.4 ± .2; medium, 108.0 ± .6; large, 178.1 ± .5 ng/ml; $P < .01$). Treatment × time × size interaction differences ($P < .01$) were primarily after the LH surge in large follicles (Table 3) and later increases in follicular progesterone representing the subsequent luteinization of an unovulated follicles in both the LH surge and no detectable LH surge groups.

Changes in follicular fluid estradiol at 1 to 6 d into the subsequent estrous cycle exhibited significant treatment × time × size interaction, primarily at day 1 in which medium- and large-sized follicles were still increased in estradiol (Table 3), probably from the prior FSH/LH stimulation and day 4 large follicles from the next follicular wave. Increases in medium-sized follicles in animals not exhibiting an LH surge at day 6 also had higher estradiol concentrations compared to follicles from animals exhibiting a prior LH surge ($P < .05$). Overall mean concentration for estradiol was not different for treatment.

Follicular prolactin concentrations were not different between animals exhibiting and not exhibiting an LH surge in the early stages of the next estrous cycle. Follicular prolactin concentrations increased over time in both treatment groups ($P < .01$; Table 3). Follicular size differences of increased prolactin concentrations were detected only in the small follicular populations on day 6 (treatment × time × size; $P < .01$). Significant differences in glycosaminoglycans ($P < .01$) were noted in relation to follicular size (small, 1862.7 ± 1.0; medium, 1113.9 ± 1.2; large, 881.6 ± .5) in which small-sized follicles had increased glycosaminoglycan concentration throughout the times monitored (Figure 5). There was a tendency for follicular glycosaminoglycans to increase over time in animals that had no detectable LH surge ($P < .05$; Table 3) whereas animals that had an LH surge were relatively stable. Animals that did not exhibit an LH surge had increased follicular glycosaminoglycan concentrations, predominantly in small and medium follicles (Figure 5).

Oocyte Recovery. Recovery of oocytes from follicular fluid of small follicles generally exceeded 90% (Tables 4 and 5; buffer wash of ≤ 4 mm diameter follicles only represents rinsing of syringe). Although animals not exhibiting an LH surge showed increased numbers of oocytes in the follicular fluid of small follicles (Table 4), when combined with oocytes from the buffer wash of the syringe, few differences were noted between treatments. Collection of oocytes in the follicular fluid of medium follicles (> 4 and ≤ 8 mm diameter) was considerably reduced as compared to small follicles (≤ 4 mm diameter) and the majority of oocytes were found in the buffer rinse of medium follicles (Table 5). Generally, animals not exhibiting an LH surge had increased numbers of oocytes from medium follicles in the buffer rinse as compared to those exhibiting an LH surge (Table 5). In large ovulatory follicles (> 8 mm diameter), animals exhibiting an LH surge had increased numbers found in the follicular fluid whereas in animals not exhibiting an LH surge, oocytes were predominantly found in the buffer flush of the follicle (Tables 4 and 5). Overall oocytes were predominantly found in the follicular fluid from animals in which an LH surge was detected and in the buffer wash of the follicle in animals in which no LH surge was detected (52.0 *vs* 34.7%; $P < .05$). Oocyte recovery was highest in small follicles (94.1 ± 1.7%) *vs* medium (68.1 ± 1.7%) or large follicles (62.7 ± 2.0%). Follicular progesterone concentrations were increased in Norgestomet-implanted animals in which no oocytes were recovered as compared to

TABLE 3. CHANGES IN FOLLICULAR HORMONES AND GLYCOSAMINOGLYCANS DURING THE SUBSEQUENT ESTROUS CYCLE IN ANIMALS EXHIBITING AN LH SURGE AND NO DETECTABLE LH SURGE DURING THE PRIOR ESTRUS.

Treatment:	LH Surge					No detectable LH surge				
Days:	1 (80)[a]	1.5 (86)	2 (44)	4 (41)	6 (284)	1 (50)	1.5 (53)	2 (44)	4	6 (96)
Follicular progesterone, ng/ml										
≤ 4 mm[b]	164.2 ± 31.8[d,e]	40.9 ± 1.9[d]	177.3 ± 83.8[d]	162.2 ± .4[d]	126.7 ± .3[d]	69.5 ± 11.5[d]	—	362.0 ± 168.0[d]	—	61.0 ± 2.2[d]
> 4 and ≤ 8 mm	492.0 ± 189.0[e]	906.8 ± 542.0[e]	200.3 ± 121.6	630.0 ± 1.1[e]	306.9 ± 1.3	95.9 ± 27.4	75.9 ± 71.1	743.3 ± 150.0	—	65.1 ± .3
> 8 mm	994.5 ± 279.1[e]	3303 ± 838.0[e]	330.1 ± 110.0	289.5 ± 4.6	494.3 ± .7	240.3 ± 148.0	170.2 ± 71.0	329.3 ± 140.1	—	539.1 ± 43.0[e]
Follicular estradiol, ng/ml										
≤ 4 mm	1.7 ± .2[d]	1.7 ± .2	1.5 ± .3	1.9 ± .3	2.3 ± .1	1.5 ± .4[d]	—	2.7 ± .7	—	2.1 ± .3
> 4 and ≤ 8 mm	5.8 ± .5[e]	2.9 ± .6	1.6 ± .9	1.2 ± .6	1.4 ± .4	1.4 ± .5	1.5 ± .6	1.4 ± .3	—	14.5 ± 3.1[e]
> 8 mm	6.3 ± 1.1	1.5 ± .3	2.0 ± 1.8	7.4 ± .6	2.0 ± 4.0	5.8 ± .9	2.3 ± .3	1.5 ± .4	—	1.2 ± .3
Follicular prolactin, ng/ml										
≤ 4 mm	[c]7.2 ± .3	10.2 ± .2	6.5 ± .3	26.7 ± .5	79.9 ± 2.8[d,e]	[c]16.5 ± 1.3	4.6 ± 3.4	6.2 ± .3	—	27.0 ± 3.3
> 4 and ≤ 8 mm	[c]8.1 ± .3	8.3 ± .4	3.2 ± .4	14.6 ± .5	23.6 ± .4[e]	[c]9.2 ± 1.7	6.5 ± .7	7.0 ± .8	—	16.3 ± .7
> 8 mm	[c]9.2 ± .4	7.6 ± .5	3.6 ± .6	23.4 ± 1.0	26.2 ± .4[e]	[c]7.4 ± .9	6.2 ± .3	5.5 ± .6	—	13.6 ± .3
Follicular glycosaminoglycans, μg/ml										
≤ 4 mm	[c]1383.3 ± 32.7[d]	1558.8 ± 30.8[d]	1573.5 ± 34.6[d]	2037.7 ± 34.6	2110.2 ± 42.2[d]	[c]1205.9 ± 141.7[d]	—	1652.2 ± 305.0[d]	—	2622.5 ± 72.3[d]
> 4 and ≤ 8 mm	1177.6 ± 155.2	919.7 ± 85.0	1319.8 ± 66.7	—	1239.3 ± 187.6	[c]1231.2 ± 150.6	1026.1 ± 86.6	[c]1210.1 ± 94.5	—	1335.2 ± 118.6
> 8 mm	962.9 ± 77.8	843.2 ± 76.9	1169.4 ± 36.0	1937.6	708.3 ± 99.1	[c]1028.5 ± 56.0	933.2 ± 23.0	[c]939.7 ± 79.0	—	1443.7 ± 66.8

[a]Day of subsequent estrous cycle after superovulation and numbers of follicles sampled ( ).
[b]Follicular size, mm diameter.
[c]Significantly changed with day.
[d]Significantly changed with follicular size.
[e]Significantly different between treatments for comparable time/size.

TABLE 4. PERCENT RECOVERY OF OOCYTES IN FOLLICULAR FLUID OF ALL FOLLICLES FROM BOTH OVARIES SAMPLED IN ANIMALS EXHIBITING AN LH SURGE, NO DETECTABLE LH SURGE, OR IN WHICH THE LH SURGE WAS SUPPRESSED WITH NORGESTOMET IMPLANTS.

Follicle size/	Hours Post Prostaglandin Injection										
Treatment	12	24	36	48	60	72	84	96	108	192	240
≤ 4 mm[a]											
LH surge	86.2 ± 6.7	84.5 ± 5.5	86.9 ± 4.9	88.3 ± 7.8	89.1 ± 5.8	84.6 ± 4.9	69.3 ± 5.5	75.2 ± 5.6	61.7 ± 8.9	68.6 ± 5.8	79.4 ± 2.2
No detectable LH surge				80.8 ± 16.0	81.4 ± 7.6	99.0 ± 10.7	94.4 ± 6.4[b]	89.7 ± 12.1[b]	76.0 ± 6.7		
Norgestomet Implants						82.5 ± 4.3	80.6 ± 4.8	84.3 ± 4.1	92.5 ± 4.2[b]		
> 4 and ≤ 8 mm											
LH surge	7.5 ± 3.5	20.9 ± 5.2	19.2 ± 4.6	22.5 ± 6.2	40.1 ± 7.6	20.7 ± 7.6	14.9 ± 6.1	28.4 ± 8.2	12.8 ± 10.5	11.6 ± 10.4	3.5 ± 5.2
No detectable LH surge				14.6 ± 10.8	14.5 ± 9.6[b]	14.2 ± 8.2	12.0 ± 9.0	28.3 ± 8.7	14.5 ± 6.4		
Norgestomet Implants						2.0 ± 4.8[b]	14.6 ± 5.3	14.6 ± 5.1	10.5 ± 4.3		
≥ 8 mm											
LH surge	20.9 ± 4.5	16.0 ± 3.8	21.1 ± 4.4	27.0 ± 4.3	53.8 ± 4.0	25.4 ± 8.1	48.0 ± 11.9	32.0 ± 9.6	31.1 ± 13.2	7.0 ± 1.8	12.5 ± 4.6
No detectable LH surge				11.5 ± 8.4	3.6 ± 6.9[b]	12.6 ± 9.6[b]	3.4 ± 7.2[b]	12.0 ± 5.8[b]	1.8 ± 8.8[b]		
Norgestomet Implants						17.1 ± 4.9[b]	11.7 ± 4.4[b]	19.9 ± 5.3[b]	4.7 ± 4.3[b]		

Small follicles were not flushed with buffer. Means ± SE represent proportion of oocytes left in the syringe (11.4 ± 1.9%) after collection of oocytes from follicular fluid. Percent recovery = numbers of oocytes found in the follicular fluid divided by total number of follicles sampled for that response group, follicular size, time post $PGF_{2\alpha}$.

LH surge *vs* no detectable LH surge or Norgestomet ($P < .05$). Overall, Norgestomet was not different from no detectable LH surge.

TABLE 5. PERCENT RECOVERY OF OOCYTES IN BUFFER WASH OF ALL FOLLICLES SAMPLED IN ANIMALS EXHIBITING AN LH SURGE, NO DETECTABLE LH SURGE, OR IN WHICH THE LH SURGE WAS SUPPRESSED WITH NORGESTOMET IMPLANTS.

Follicle size/	Hours Post Prostaglandin Injection										
treatment	12	24	36	48	60	72	84	96	108	192	240
≤ 4 mm[a]											
LH surge	8.0 ± 7.9	11.6 ± 6.6	11.0 ± 5.3	6.9 ± 7.2	11.5 ± 6.9	6.7 ± 5.8	7.7 ± 6.5	27.3 ± 6.7	17.3 ± 10.5	30.0 ± 6.9	19.8 ± 2.6
No detectable LH surge				16.3 ± 19.0	13.3 ± 9.0	1.0 ± 12.6	4.4 ± 7.6	1.0 ± 14.3[b]	10.3 ± 8.0		
Norgestomet Implants						10.8 ± 5.1	21.6 ± 5.7	16.1 ± 4.9	6.4 ± 4.9		
> 4 and ≤ 8 mm											
LH surge	50.0 ± 4.1	45.8 ± 6.1	43.2 ± 5.3	42.2 ± 7.4	40.5 ± 9.0	58.7 ± 9.0	34.8 ± 7.2	24.9 ± 9.7	45.0 ± 12.4	80.9 ± 12.3	45.0 ± 6.2
No detectable LH surge				59.3 ± 12.8	55.9 ± 11.5	42.7 ± 9.8	62.0 ± 10.7[b]	59.5 ± 10.3[b]	57.0 ± 7.7		
						68.0 ± 5.7	58.8 ± 6.3[b]	59.4 ± 6.1[b]	58.5 ± 5.0		
≥ 8 mm											
LH surge	58.9 ± 5.4	57.9 ± 4.5	42.6 ± 5.3	36.2 ± 5.2	26.3 ± 4.8	38.2 ± 9.2	9.3 ± 14.1	2.4 ± 11.4	28.4 ± 15.6	45.5 ± 21.6	22.5 ± 5.5
No detectable LH surge				73.7 ± 9.9[b]	69.8 ± 8.2[b]	58.6 ± 11.5	44.6 ± 8.6[b]	53.4 ± 6.9[b]	34.8 ± 10.4		
Norgestomet Implants						48.7 ± 5.8	66.6 ± 5.2[b]	48.3 ± 6.3[b]	57.9 ± 5.0		

[a]Small Follicles were not flushed with Buffer. Means ± SE represent proportion of oocytes left in the syringe (11.4 ± 1.9%) after collection of oocytes from follicular fluid.
[b]LH *vs* no detectable LH or Norgestomet ($P < .05$).

animals in which no LH surge was detected (344.7 ± 63.3 *vs* 107.3 ± 15.3 ng/ml, respectively; $P < .05$). Otherwise, follicular estradiol prolactin and glycosaminoglycans were not different between no detectable LH surge and Norgestomet-implanted animals. No trend was detected with animals that exhibited an LH surge or with no LH surge in relation to follicular progesterone concentration and no recovery of oocytes. No differences in follicular estradiol or prolactin levels were detected in relation to recovery of oocytes in animals in which an LH surge and no LH surge were detected. Overall follicular progesterone and glycosaminoglycans were increased when oocytes were collected from the follicular fluid as compared to the buffer rinse of the follicle (progesterone 234.1 ± 19.6 *vs* 106.6 ± 13.1 ng/ml; $P < .01$), but more likely reflected developmental changes associated with general maturation of the follicles and treatment effects than any aspect of recovery of the oocytes (Figures 2 and 5).

Oocyte Viability. Oocyte viability increased with follicular size in animals exhibiting an LH surge or in which no LH surge was detected, but decreased in viability with follicular size in the group in which the LH surge was suppressed with Norgestomet implants (Table 6). Oocytes from Norgestomet-implanted animals were 75% degenerate whereas oocytes from animals exhibiting an LH surge were 75% viable. Viability of oocytes was better in medium (> 4 and ≤ 8 mm diameter) and large follicles (> 8 mm diameter) from animals exhibiting an LH surge than those in which no LH surge was detected or the LH surge was suppressed with Norgestomet implants (Table 6). In both treatments that had no LH surge, follicular progesterone concentrations were increased in follicles with degenerate oocytes (Table 7), particularly in the later stages of the estrual period (72–108 hr, Figure 2). Follicular fluid estradiol concentrations were increased in follicles producing viable oocytes in both animals exhibiting an LH surge and those not exhibiting an LH surge, primarily in medium and large follicles (Figure 3). Although follicular fluid prolactins were increased in follicles producing degenerate oocytes (15.8 ± 1.2 *vs* 13.4 ± 1.1 ng/ml), few differences were noted between treatments or quality of oocytes (Figure 4). Follicular glycosaminoglycans were increased in follicles producing degenerate oocytes in the LH surge treatment group (Table 7; Figure 5). Overall increased concentrations of glycosaminoglycans in treatments that did not exhibit an LH surge (no detectable LH or Norgestomet implanted; Figure 5) may also be related to the decreased viability of oocytes in these treatment groups and subsequently mask differences detected in the LH surge group (1203.6 ± 21.9 ($P < .1$) and 1221.6 ± 10.8 ($P < .01$) vs 1148.9 ± 24.9 μg/ml, respectively).

DISCUSSION

There is an important link between the FSH-induced estrogen response to the quality and quantity of follicles that eventually leads to the production of high quality oocytes (3, 29). The follicular estradiol and peripheral estradiol trends and concentrations are quite different in animals exhibiting an LH surge and those that do not (Figures 1 and 3). Not only is the production of estradiol dependent on granulosa response to FSH (3) but also LH (Figure 3) is required for maximal production of estradiol (30, 31). Deviations from low follicular progesterone and high estradiol concentrations can alter the estradiol surge and LH peak with fewer oocytes maturing to useful stages (32–34) although other work shows high levels of progesterone and low levels of estradiol are best for in vitro maturation of oocytes (35). By 72 hr after $PGF_{2\alpha}$ injection, follicular estradiol concentrations have decreased (21, 36). Without the preovulatory stimulation of LH, estradiol concentrations do not attain ovulatory values in follicles (Figure 3b). Follicular estradiol concentrations in the subsequent estrous cycle (days 1 to 6) are low and most likely represent the effect of various stages of follicular atresia (Table 3), but increases in estradiol in medium-size follicles in animals that did not exhibit an LH surge

TABLE 6. PERCENT VIABLE OOCYTES FROM BOTH OVARIES IN ANIMALS THAT EXHIBITED AN LH SURGE, NO DETECTABLE LH SURGE, OR IN WHICH THE LH SURGE WAS SUPPRESSED WITH NORGESTOMET IMPLANTS.

	Hours post prostaglandin injection								
Follicle size/treatment	12	24	36	48	60	72	84	96	108
≤ 4 mm (n = 716)									
LH surge	75.6 ± 11.6	39.9 ± 8.6	59.2 ± 7.8	67.9 ± 15.8	28.5 ± 9.8	29.1 ± 12.2	42.1 ± 12.1	66.4 ± 11.3	77.9 ± 20.0
No detectable LH surge				85.7 ± 4.1	52.4 ± 18.2	90.7 ± 16.9[a,b]	7.3 ± 9.0[a,b]	31.5 ± 18.7	37.9 ± 12.8[a]
Norgestomet Implants						54.5 ± 5.8[a]	37.6 ± 5.8	21.4 ± 5.6[a]	24.2 ± 6.4[a]
>4 and ≤ 8 mm (n = 441)									
LH surge	54.9 ± 6.6	65.7 ± 8.2	29.3 ± 8.7	73.8 ± 12.6	84.6 ± 9.8	97.2 ± 10.2	80.9 ± 12.1	71.0 ± 13.8	17.4 ± 19.6
No detectable LH surge				28.6 ± 16.7	57.5 ± 16.3	68.0 ± 14.5[b]	63.5 ± 14.4[b]	69.8 ± 18.4[b]	65.7 ± 11.9[b]
Norgestomet Implants						36.7 ± 6.7[a]	29.2 ± 7.1[a]	22.5 ± 7.2[a]	20.8 ± 6.0
> 8 mm (n = 528)									
LH surge	53.7 ± 7.1	57.7 ± 5.5	82.6 ± 6.8	67.9 ± 9.8	66.6 ± 5.4	96.6 ± 13.8	98.7 ± 22.3	70.5 ± 22.9	0.0
No detectable LH surge				68.5 ± 10.8	76.6 ± 9.7	72.2 ± 14.3[b]	22.9 ± 13.3[a]	56.4 ± 9.1[b]	18.5 ± 26.0
Norgestomet Implants						16.7 ± 7.5[a]	19.4 ± 5.6[a]	19.7 ± 7.8	19.5 ± 6.5

[a]LH surge *vs* no detectable LH surge or Norgestomet implant ($P < .05$).
[b]No detectable LH surge *vs* Norgestomet implant ($P < .05$).

TABLE 7. FOLLICULAR FLUID DIFFERENCES OF ESTRADIOL, PROGESTERONE, PROLACTIN, AND GLYCOSAMINOGLYCANS IN VIABLE AND DEGENERATE OOCYTES.

Oocytes	Estradiol[a] (n)	Progesterone[a] (n)	Prolactin[a] (n)	Glycosaminoglycans[b] (n)
LH surge detected				
Viable	33.5 ± 2.8[c] (237)	154.7 ± 26.2[d] (261)	14.0 ± 1.1 (284)	1038.4 ± 24.1[c] (240)
Degenerate	28.9 ± 2.5 (212)	251.3 ± 23.4 (224)	15.8 ± 1.2 (235)	1091.7 ± 21.6 (199)
No LH surge detected				
Viable	40.5 ± 5.1 (186)	48.2 ± 36.6[d] (205)	12.7 ± 1.2 (219)	1069.6 ± 55.0 (191)
Degenerate	35.0 ± 4.7 (173)	103.5 ± 39.6 (181)	15.8 ± 2.4 (195)	1024.0 ± 22.4 (163)

[a]Mean ± SE, ng/ml.
[b]μg/ml.
[c]$P < .05$.
[d]$P < .01$.

in the prior cycle may represent the first wave of follicular growth in the next estrous cycle.

Increased progesterone impacts heavily on follicular function in that LH stimulation required for steroid synthesis is altered and ovulation inhibited as in Norgestomet-implanted animals (Table 3). Reduced fertility when progestin implants are utilized to synchronize animals to estrus (37, 38) may be related to the progestin effects on LH stimulation that accompanies follicular development prior to ovulation. As 20% of the animals sampled did not demonstrate an LH surge or subsequent ovulation, it was originally hypothesized that enough progestin may be present (peripheral or follicular) to either inhibit the LH surge or alter its pattern enough where ovulation, oocyte quality, and fertility would be decreased. Synthetic progestins do not mimic corpus luteum function and altered profiles of LH have been documented from synthetic progestins, which leads to abnormal development of follicles (39). The increased follicular progesterone concentrations in animals that did not exhibit an LH surge compared to Norgestomet-implanted animals in which the LH surge was suppressed (Table 2; no detectable LH surge *vs* Norgestomet implanted, 242.3 ± 36.3 *vs* 86.7 ± 6.4 ng/ml; $P < .01$) indicates some LH stimulation, even though inadequate for ovulation. In Norgestomet-implanted animals, little LH stimulation of progesterone synthesis was noted (Table 2). It is thought that increased progesterone concentrations from either the LH contamination in the FSH preparation or possibly stress of handling produces abnormal LH profiles (33) and prevents the estradiol-induced LH surge in conjunction with producing asynchrony of oocyte maturation (32, 40). Animals in this study that had no detectable LH surge had neither increased peripheral (Figure 1) nor follicular progesterone (Figure 2) concentrations that might indicate a possible reason the LH surge was suppressed. After the LH surge ($\bar{x}$ = 42 hr post $PGF_{2\alpha}$), progesterone has started to increase in follicles (84 to 108 hr) to indicate initiation of the luteinization process and subsequent atresia of unovulated follicles (Figure 2a). Increases in follicular progesterone concentrations (96 to 108 hr) and the early days of the subsequent estrous cycle from animals not exhibiting an LH surge most likely indicate unovulated follicles undergoing atresia (Figure 2b; Table 3; 41) but are considerably lower than follicular progesterone from animals exhibiting an LH surge. Oocytes collected from these follicles for in vitro fertilization would hardly be expected to be high quality oocytes as lack of LH stimulation for oocyte maturation is associated with degenerate oocytes in cattle (42) and the majority of these oocytes were degenerate. Alternatively, high follicular fluid progesterone concentrations are related to increased probability of oocyte recovery after aspiration (4).

Bovine prolactin concentrations decreased with increases in follicular size and as the follicular phase advanced, which is similar to reports in humans (43–45) but contrary to the report of Henderson et al. (46) with bovine follicles. The decline in follicular pro-

lactin comes 18–24 hr after the LH surge is probably not directly induced by the LH surge although pituitary release of LH at ovulation may have also caused a coordinate release of prolactin. Prolactin has been shown to inhibit progesterone biosynthesis (16, 47, 48), estrogen production (17, 49, 50) and inhibit luteinization of granulosa cells (19). Thus, an inverse relationship between follicular progesterone and prolactin concentrations has been documented (15, 46, 51, 52) and supported in this study. Contrary to other results (53, 54), Bevers et al. (55) found no prolactin receptors in bovine granulosa cells and bromocriptine treatment did not alter the ovulatory development of follicles. Dusza et al. (56) noted no effect on peripheral progesterone concentrations after prolactin administration to gilts and sows, but bromocriptine reduced prolactin levels and growing follicles in sheep (57). Hyperprolactinemia can interfere with ovarian function and results in infertility in women (58, 59). Porcine and rat granulosa cells are stimulated by prolactin in vitro (60, 61) but differences in response may heavily depend upon developmental maturity of the follicle (16, 62). There may be differences of responses in vivo as compared to in vitro as follicular steroids and prolactin may modulate granulosa cell function (63).

Prolactin will also inhibit follicular estradiol production (17, 49, 50, 64), but estradiol will stimulate pituitary prolactin synthesis (65). Prolactin release and synthesis are estrogen dependent. Thus, a positive correlation of follicular/peripheral estrogens and prolactin may also be expected (66, 67). In this study, follicular prolactin concentrations were inversely related to progesterone concentrations (Figure 2b) and paralleled estrogen concentrations (Figures 3 and 4). In the subsequent cycle (days 2 to 6; Table 3) prolactin concentrations increase with time and were highest in concentration in the smallest follicles. Prolactin has a modulatory role in small follicles but few effects on larger follicles (62) and may explain the increases in prolactin in small follicles during the subsequent estrous cycle (Table 3). Inhibitory effects of prolactin on granulosa synthesis of estrogens seems to be from inhibiting the induction of the aromatase enzyme via FSH stimulation (64, 68), thus increased prolactin in small follicles may limit estrogen synthesis in small follicles in the subsequent cycle (Table 3). As well as the effects of prolactin on follicular development and granulosa function, the role prolactin plays in oocyte maturation may be inhibitory or stimulatory (69, 53, 70). Increased follicular prolactin inhibits plasminogen activity and subsequently interferes with rupture of ovulatory follicles (71), thus increased concentrations of prolactin noted in small follicles seems functionally valid. It is unknown whether prolactin exerts its anti-gonadal effect at the ovarian level (18) or hypothalamic-pituitary axis (72) but hyperprolactinamemia is associated with reduced ovarian function (44, 59, 73).

As noted by other reports (14, 74, 75), glycosaminoglycans decrease with increases in follicular size (Figure 5). Low concentrations of glycosaminoglycans have been reported to be associated with follicles with high estrogen (11, 12) and low progesterone content (13, 14) and synthesis of follicular glycosaminoglycans is partially under the control of progesterone (76, 77). Also, HCG/LH inhibits glycosaminoglycan synthesis (76) and FSH stimulates synthesis (78–80). Glycosaminoglycans tend to be increased in animals that did not exhibit an LH surge (Figure 5b) and low glycosaminoglycans were associated with increased estradiol (LH surge animals). Conversely, animals that exhibited high follicular estradiol had also increased follicle progesterone content, partially related to the LH surge; thus, the control aspects of glycosaminoglycan synthesis of the follicle is not clear in response to the superovulation treatment. Follicular glycosaminoglycans in animals that had the LH surge inhibited with a progestin implant were comparable to those that exhibited no LH surge (Table 2). Follicular glycosaminoglycans are playing an ever enlarging role in follicular and oocyte maturation (81–83) and as binding proteins for growth factors (9) may be a regulator of follicular

development (11). Effects of follicular glycosaminoglycans are not limited to granulosa cells as concentrations of glycosaminoglycans may have an effect on oocyte maturation and subsequent fertilization rates. When the major component of follicular glycosaminoglycans, dermatan sulfate (> 75%) exceeds an 800 μg/ml concentration, in vitro fertilization rates decrease (84). Increased concentrations of glycosaminoglycans are associated with atretic follicles. Concentrations of glycosaminoglycans in small follicles (Figure 5) and in follicles of the subsequent estrous cycle (days 1 to 6; Table 3) would be assessed to be atretic (follicular fluid exceeds 800 μg/ml of glycosaminoglycans).

Oocyte viability from animals in which no LH surge was detected fell between those that exhibited an LH surge and those in which the LH surge was suppressed with progestin implants and thus may indicate some LH stimulation even though no LH surge was detected or ovulations noted. Deviations in normal progesterone on LH profiles during the estrual period leads to poor egg/embryo quality (32). The mean number of degenerating oocytes were less if collected while serum estradiol levels were increasing (85), but this study showed viability was good in medium and large follicles long after follicular estradiol concentrations decreased. High progesterone and low estrogen concentrations in follicular fluid are also indicative of granulosa cells that are not undergoing cell division in the preovulatory follicle (86) and are probably in some stage of luteinization/atresia. The increased numbers of degenerate oocytes (medium and large follicles; Tables 6 and 7) were related to increased follicular progesterone content primarily at the later stages of the estrual period (72 to 108 hr post $PGF_{2\alpha}$ injection; Figure 2). Small follicles from animals that exhibited an LH surge had surprising poor oocyte viability 48 to 72 hr post $PGF_{2\alpha}$ injection (Table 6). This may be related to aspects of asynchrony of activation of cumulus and oocyte maturation (87) as compared to animals with no detectable LH surge in which oocytes would be stabilized as immature.

Although different mechanical techniques have been analyzed in relation to procuring oocytes (follicular aspiration, slicing of ovary, and collagenase treatment), aspects of follicular hormones, sizes, and time of the estrous period on recovery are largely unknown. The complexity of events leading to the requirement of recovery of a viable oocyte for in vitro fertilization is outlined by Greer et al. (4). Heifers exhibiting an LH surge produce an increased recovery rate of oocytes (32, 88). This is only partially supported in this study in that oocytes were primarily collected in the follicular fluid of animals exhibiting an LH surge and the buffer wash from animals with no detectable LH surge (Tables 4 and 5), which may indicate differences in release of oocytes. Total numbers of follicular oocytes recovered (follicular fluid + buffer rinse of follicles) did not differ between treatments. As follicular size increased in animals exhibiting an LH surge, oocytes were predominantly found in the follicular fluid from aspiration, but in animals with no detectable LH surge, oocytes were found in the buffer rinse of the follicle. Callesen et al. (40) suggest that low recovery rate from animals without an LH surge is due to increased oocyte fragility from the abnormal follicular microenvironment (abnormal follicular steroids associated with degenerate follicles) but intact, viable oocytes were recovered with buffer washes of follicles (Table 5) in animals that had no detectable LH surge and supports differences in release of the oocyte from the stimulation of LH. Follicular fluid progesterone content was related to recovery of an oocyte. As follicular fluid progesterone content increased, oocyte recovery decreased as reported by Greer et al. (4).

In these experiments, the importance of the LH surge on follicular maturation, steroidogenesis, synthesis of glycosaminoglycans and ovulation was delineated in conjunction with oocyte recovery and viability. Inhibition of the LH surge with progestin implants (Norgestomet) produces similar results to animals in which no LH surge was detected, although progestin implants were more effective at reducing follicular proges-

terone concentrations and decreasing recovery and viability of oocytes. Increases in follicular prolactin and glycosaminoglycans in the early days of the subsequent estrous cycle may be indicative of follicular atresia or lack of gonadotropin stimulation of follicles during this phase of the estrous cycle. Follicular glycosaminoglycan concentrations have been reported as a marker for follicular atresia (11, 13). Viability and recovery of oocytes were highly associated with the LH surge.

The elimination of the LH surge either normally or artificially with progestin implants results in different follicular secretory patterns of steroids and glycosaminoglycans with an end result which may be an increase in poor quality oocytes and lowered recovery. The decrease in fertility associated with estrous synchronization with progestins (even though synthetic progestins are thought to be cleared prior to estrus) as opposed to prostaglandins may be related to the altered gonadotrophin effect on follicular development, estrogen/progesterone synthesis, and ovulation.

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[1]Mention of names is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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